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Detection of Genetic Thrombophilia (Fa V Leiden & PT20210 mutation) using GeneXpert Technology with IQCP Implementation

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Detection of Genetic Thrombophilia (FV Leiden and PT 20210) Using GeneXpert Technology with IQCP Implementation

By

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A culminating project submitted to the faculty of Dominican University of California in partial fulfillment of the requirements for the degree of Master of Science in Clinical Laboratory Sciences

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ABSTRACT

Individuals having Genetic Thrombophilia pose a higher risk of having a thrombotic event. It is crucial to determine these gene variants to prevent a possible episode of thromboembolism. With the current PCR method, it involves individual processing of DNA isolation, amplification, and detection using three (3) different instruments resulting to an increased turnaround time of 5 to 7 days and additional staff utilization. It is performed by repetitive manual sample pipetting and preparation of reagent master mixes in small vials. Results interpretations are entered manually to a worklist built initially for final verification. These processes increase the risk of staff injury and potential result error that could impact patient management.

The introduction of the GeneXpert technology by Cepheid will aid in the prevention of staff injury from repetitive motion, improve the turnaround time and eliminate potential risk of error. This test system performs DNA isolation, amplification and detection within a cartridge kit that will decrease instrument preventive maintenance costs and personnel hands-on utilization. Furthermore, an individualized quality control plan (IQCP) will be implemented after risk assessment of the pre-analytic, analytic and post-analytic phase of testing to customize quality control frequency ensuring the accuracy of test results upon approval by the Laboratory director.

A combination of 104 whole blood samples of sodium citrate (83) and EDTA (21) that were previously tested with the current method was used for the validation study. Fifteen (15) of the whole blood citrate samples were frozen after testing to confirm the manufacturer's claim of an alternative sample. Seventy-nine (100%) whole blood citrate samples and twenty-one (100%) whole blood EDTA samples were correlated with the results of the current PCR method.

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Life is a bit bearable with the people who inspire me. Thank you for your wisdom and strength. Lastly, Thank you, Lord God. I did it.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

APCR- Activated Protein C Resistance

APL- Anti Phospholipid Antibodies

CAP- College of American Pathologist

CLIA- Clinical Laboratory Improvement Amendments

DVT- Deep Vein Thrombosis

EDTA- Ethylene Diamine Tetra Acetic Acid

FVL= Factor V Leiden

IQCP- Individualized Quality Control Plan

LA-Lupus Anticoagulant

PCR- Polymerase Chain Reaction

QA- Quality Assessment

QCP- Quality Control Plan

RA- Risk Assessment

INTRODUCTION

Genetic thrombophilia is the most common hereditary disorder that increases the likelihood occurrence of thrombosis. It can be identified with a significant minority of patients with venous thromboembolism and in most patients with well-known thrombotic episodes (Murin, Marelich, Arroliga, & Matthay, 1998).

There are also other endogenous anticoagulant deficiencies such as Protein C, Protein S and Antithrombin that have been determined to cause hypercoagulable state, but the incidence is low with patients with familial thrombosis. As a result of extensive research and study, it was discovered that resistance to activated protein C (APC) is the most common genetic risk factor for venous thrombosis. It is caused by a single point mutation in the factor V gene. A gene that transcribes the protein called coagulation factor V. These coagulation factors is a group of related proteins that make up the coagulation system that is responsible for the formation of blood clots after an injury and trigger blood vessel repair (Genetics Home Reference, n.d.). Another important type of hereditary thrombophilia is a variant that occurs in the prothrombin gene at allele 20210 (PT20210), its prevalence and association of thrombosis is not as high compared Factor V Leiden but is significant. The most common clinical manifestation is deep vein thrombosis (DVT) thus, the increased ability to determine underlying risk factors with thrombotic patients will enable to perform immediate testing with these genetic variants.

The detection of the Factor V Leiden mutation and PT20210 allele are best performed using point mutation real-time PCR analysis due to technique simplicity and allows differentiation from rare variants (Peter C. Cooper & Anne C. Goodeve, 2012).

ACTIVATED PROTEIN C RESISTANCE (FACTOR V LEIDEN)

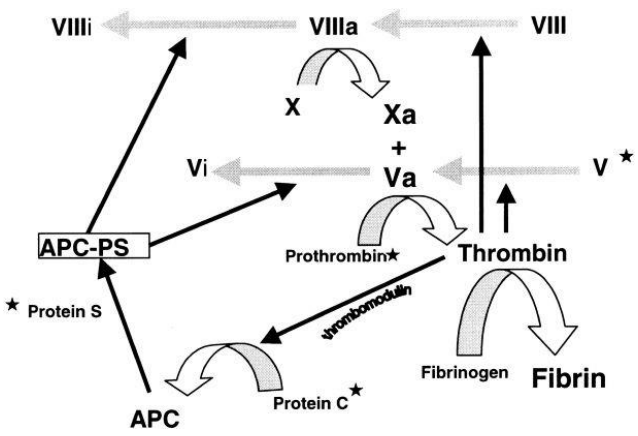

FIGURE 1 SIMPLIFIED COAGULATION CASCADE & PROTEIN C ANTICOAGULATION SYSTEM

The factor V allele is resistant to the proteolytic effect of Protein C in patients with activated protein C resistance. This resistance is due to the transition of guanine to adenine at nucleotide position 1691 (G1691), a gene product called Factor V Leiden. The single point mutation in the Factor V gene resulting in a substitution of arginine with glutamine at position 506 in one of the protein cleavage sites and renders activated Factor V resistant to cleavage and the inability to deactivate Factor V and Factor VIII in the coagulation cascade causing increased formation of thrombin resulting to clot formation. (Figure 1).

The presence of this mutation will result in a 5-10fold increase for heterozygote carriers and approximately 50 -100fold in homozygotes risk of venous thrombosis which occurs 20-60% of APC resistant individuals. The FV mutation is prevalent from 1-15% in a population of Caucasian origin (Zöller, Hillarp, Berntorp, & Dahlbäck, 1997).

PROTHROMBIN 20210A MUTATION

The activation of Factor V and Factor VIII by precursor prothrombin converts fibrinogen to fibrin. It has been identified the transition from guanine (G) to adenine (A) at the last nucleotide position 20210 in the 3'- untranslated region of the gene (figure 2) that is associated

FIGURE 2 G (20210) A PROTHROMBIN GENE

with an increased risk for venous thrombosis. Having the 20210A allele causes marked increase levels of plasma prothrombin. (Poort SR, 1996)

Heterozygotes have a 2-5fold increase risk of thrombosis and with the presence of other forms of thrombophilia will significantly increase thrombotic risk. Homozygotes are rarely occurring in less than 1% of the population.

Historically, laboratory testing for thrombophilia focuses on the detection of the endogenous anticoagulant deficiencies of Protein C, Protein S and Antithrombin, dysfibrinogenemia and antiphospholipid antibodies (APA)/lupus anticoagulants (LA) but for the past decade, venous thromboembolism has been so complex that because of its heterogeneity, diagnosis is dependent on both acquired and genetic factors. In this regard, the mutation determination of FV Leiden and PT20210 gene is highly significant in the pathogenesis of DVT in combination with acquired factors such as trauma, pregnancy, surgery, age, weight, etc.

Coexistence of both mutations of Factor V Leiden and PT 20210 are found in 10% of the population with predetermined thrombotic episodes. (Vicente R et al, 1999)

MATERIALS AND METHODS

INSTRUMENTATION

FIGURE 3 GENEXPERT SYSTEM

An FDA approved, closed platform GeneXpert system by Cepheid (Figure 3) that performs qualitative real-time PCR for automated detection and genotyping of Factor V Leiden and PT20210 alleles directly from whole blood samples. The test is intended to perform DNA isolation, amplification, and detection in a cartridge kit with a turnaround time of 30 minutes for each testing. A sample volume of 50ul of whole blood is dispensed straight into the bottom of the cartridge. Each cartridge (Figure 4) includes internal quality controls and contains freeze-dried beads with necessary components for PCR such as DNA polymerase, nucleotides, primers and scorpion probes. Through the PCR cycles, the specific binding of the probe sequence to the target mutation detected at real time and allows the software to report out both FV and PT20210 in approximately thirty-two (32) minutes.

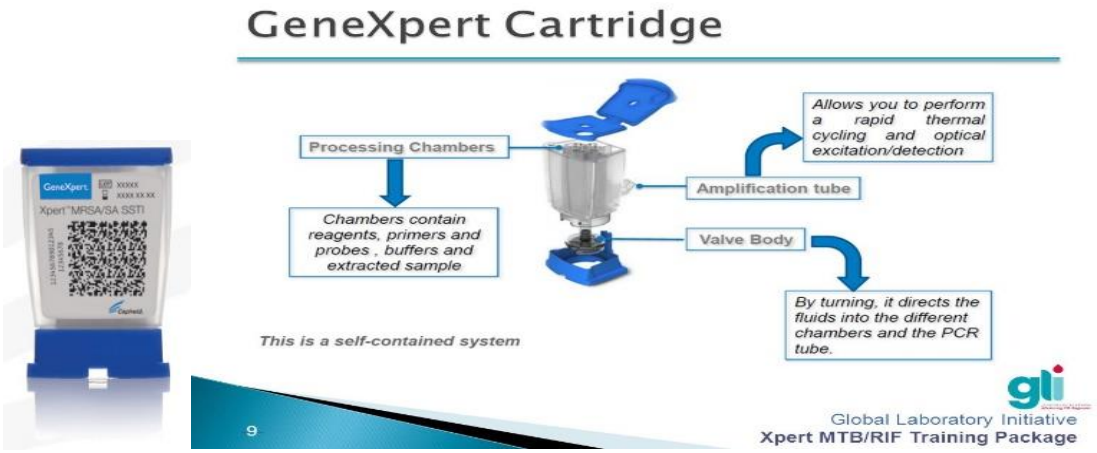

FIGURE 4 CARTRIDGE KIT

VALIDATION STUDY

A combination of 104 whole blood of sodium citrate and EDTA samples that were previously tested using current invader assay method was processed with the GeneXpert technology system. Most of these samples were stored at 2-8C and stable for 15 days and some samples were stored at room temperature with a 24hour stability. Additionally, 15 frozen citrate samples were tested to verify its suitability for testing as recommended by the manufacturer.

Precision studies consist of four (4) Sodium citrate samples consisting of one (1) normal sample for both FV Leiden and PT20210, one (1) heterozygous sample for PT20210, one (1) heterozygous sample for FV Leiden and one (1) homozygous sample for both FV Leiden and PT20210. These 4 samples were run in duplicate for 5 days.

Correlation studies were performed with eighty-five (85) whole blood samples, a combination of sixty-four (64) citrate tubes and twenty-one (21) EDTA samples. The samples included two (2) homozygous FV Leiden, one (1) homozygous PT20210, thirty-seven (37) Heterozygous FV Leiden, thirty-one (31) normal FV Leiden, seventeen (17) heterozygous PT20210 and fifty-one (51) normal PT20210. Fifteen (15) frozen citrate samples were also tested for possible consideration of using this type of sample for testing as stated by the manufacturer.

INDIVIDUALIZED QUALITY CONTROL PLAN

According to the Clinical Laboratory Improvement Amendments (CLIA) of 1988, laboratories are required to have Quality Control procedures to monitor the accuracy and reliability of test results. A minimum of 2 levels of controls must be performed every eight hours of patient testing. IQCP provides a foundation for an alternative quality control (QC) program that would allow laboratories, after appropriate assessment, the choice to implement a

customized QC plan for specific tests utilizing internal control systems. By performing the steps of IQCP, it will evaluate the potential sources of error in the three (3) phases of testing and establish an appropriate QC and best practices to prevent possible errors. After the evaluation process, other potential sources of error might be determined and that may require additional QC activities resulting in a more comprehensive QC program.

IQCP consists of 3 parts: Risk assessment (RA), Quality control plan (QCP) and Quality Assessment (QA).

1.Risk Assessment (RA)

Identification and evaluation of the risk that occurs in the preanalytical, analytical and post-analytical phases of testing. There are six (6) components that need to be evaluated for Risk assessment for potential sources of error and possible failures. These are Specimen, Test System, Reagents, Environment, Testing personnel, and Test results.

Lists of information to conduct Risk Assessment (RA)

- Laboratory policies and procedures
- CAP Checklists- Common Checklist and Molecular Pathology
- Manufacturer's package inserts (intended use, reagent, QC frequency, maintenance, environment, et al)
- Method Validation records
- Laboratory records (QC, PT, New lot verification, Maintenance)
- Error correction and Provider complaints
- Personnel Training and competency records

Based on the information listed, a table summary of Risk assessment (Table 7) was established for possible sources of error or failures consisting of the six (6) elements in relation to the different phases of testing. It is evaluated according to Risk level (Table 1) and Risk acceptability (Table 2).

TABLE 1 DETERMINATION OF RISK LEVEL

Frequency of Occurrence	Severity of Harm
Unlikely (once every 2-3 years)	Negligible (temporary discomfort)
Occasional (once every year)	Minor (temporary injury; not requiring medical intervention
Probable (once per month)	Serious (impairment, requiring medical intervention
Frequent (once a week)	Critical (life-threatening consequences)
*Unknown (detectable but the frequency is unknown	
**Undetectable (unable to detect)	

*Unknown = Frequency of occurrence is unknown. No documentation/data collected.

**Undetectable = Unable to detect error unless by direct observation. (e.g. improper collection)

TABLE 2 RISK ACCEPTABILITY MATRIX

Probability of Harm	Negligible	Minor	Serious	Critical
Frequent	Not Acceptable	Not Acceptable	Not Acceptable	Not Acceptable
Probable	Acceptable	Not Acceptable	Not Acceptable	Not acceptable
Occasional	Acceptable	Acceptable	Acceptable	Not acceptable
Unlikely	Acceptable	Acceptable	Acceptable	Acceptable
Unknown	Not Acceptable	Not Acceptable	Not Acceptable	Not Acceptable
Undetectable	Not Acceptable	Not Acceptable	Not Acceptable	Not Acceptable

2. QUALITY CONTROL PLAN

A Quality Control Plan (QCP) describes practices, procedures, and resources needed by the laboratory to ensure the quality of a testing process. The QCP includes measures to assure the

accuracy and reliability of test results, and that the quality of testing is adequate for patient care. The QCP must provide for immediate detection of errors that occur due to test system failure, adverse environmental conditions, and operator performance. It must also monitor, over time, the accuracy and precision of test performance that may be influenced by changes in the specimen, test system, reagent, environment, or variance in operator performance.

This QCP is fulfilled through the creation of written standard operating procedures for instrumentation, maintenance, quality assurance and reviews involving historical quality control review, historical proficiency testing review, test system information and all the information used to conduct the risk assessment.

Each cartridge contains internal controls that check every step of the assay that validates the system, test reagents, sample, lysis, amplification and integrity of the cartridge itself. External controls were purchased from Maine molecular Quality control Inc and were run thru a 31-day period (College of American Pathologist, Common checklist, n.d.). It is used to verify the accuracy and precision of the Cepheid GeneXpert analyzer. In an unopened bottle of external controls, it will last until the indicated expiration date when stored at 2C – 8C. In an open bottle, the controls were stable for 30 days. In the event of any QC failure, QC must be repeated with appropriate documentation and corrective action performed. A patient look-back will be performed if QC continues to fail and will immediately be addressed to Manufacturer's technical support for evaluation. For repeated results that are not in agreement, an error correction report will be performed, appropriate investigations and corrective actions will be implemented to verify instrument performance and accuracy of results.

3. QUALITY ASSESSMENT (QA)

Quality assessment is a continuous process of monitoring the effectiveness of the Quality control plan for error prevention and detection. QCP and QA will be updated when new sources of errors or failures are identified. Non-conformance and/or procedure deviation will be addressed with affected personnel by the laboratory management for appropriate corrective action(s). A review system mechanism will be established that details ongoing activities for monitoring the effectiveness of the QCP. The mechanism of Quality assurance includes:

- Verification of new lot reagent kit -test with 5 patient samples paralleled against a current lot of reagent with results within acceptable criteria.
- Training of personnel- completed all the applicable training checklists and comprehension of test procedures, signed off by Quality supervisor/manager
- Competency Assessment- performed at initial orientation, after 6 months and annual evaluation.
- Proficiency Testing- Performance of PT survey (TPM from CAP) includes handling by staff and PT failure investigation if needed.
- Error Correction reports (ECR) -Refer to the specific binder
- Medical record or Test error correction request
- Physician inquiries- document any clinician concern/or feedback
- Population statistics- Monthly report compared with historical statistics and maintained in quality control indicator folder. Acceptable criteria are 2 SD of historical results.

RESULTS

The precision or reproducibility study consisted of four (4) sample types: one (1) sample normal for both FV Leiden and PT20210, two (2) samples for each heterozygous FV Leiden and PT20210 and one (1) homozygous type for both FV Leiden and PT20210. These samples were run in duplicate for 5 days and were all 100% in agreement with the expected result (Table 3). For the accuracy study, a combination of sixty-eight (68) Sodium Citrate and EDTA samples were used for correlation, all results obtained were 100% in agreement with the results of the previous method. (Table 4)

Table 3 Precision Summary in duplicates x 5 days

Specimen ID	Obtained result	Expected Result	% Total Agreement
Normal (FVL /PT20210	Normal/Normal	Normal/Normal	100% (10/10)
Heterozygous (FVL)	Heterozygous	Heterozygous	100% (10/10)
Heterozygous (PT20210)	Heterozygous	Heterozygous	100% (10/10)
Homozygous (FVL/PT20210)	Homozygous/ Homozygous	Homozygous/ Homozygous	100% (10/10)

TABLE 4 CORRELATION SUMMARY

Specimen	Obtained result	Expected Result	% Total agreement
Homozygous (FVL)	Homozygous (2/2)	Homozygous	100%
Homozygous (PT20210)	Homozygous (1/1)	Homozygous	100%
Heterozygous (FVL)	Heterozygous (37/37)	Heterozygous	100%
Heterozygous (PT20210)	Heterozygous (17/17)	Heterozygous	100%
Normal (FVL)	Normal (31/31)	Normal	100%
Normal (PT20210)	Normal (51/51)	Normal	100%

With the additional fifteen (15) frozen samples, thirteen (13) samples correlated with previous verified results, two (2) samples were interpreted as invalid probably due to gross hemolysis and/or loss of integrity. A frozen sample is unlikely to be used primarily because serum or plasma is the preferred sample for testing.

The overall accuracy and precision performance of GeneXpert technology using whole blood sodium citrate and EDTA samples demonstrate 100% agreement with the predicate method, Invader chemistry. Cepheid GeneXpert yielded a 98.06% performance agreement with the inclusion of frozen samples for a possible alternative sample type but determined not to be used.

IQCP SUMMARY

The implementation of IQCP for the detection of both the FV Leiden and PT20210 mutation using GeneXpert technology will assure the accuracy of the test result through the assessment of the pre-analytical, analytical, post-analytical phases of testing. The risk assessment outcome involving the six (6) components suggest probable risk occurring during pre-analytical testing such as mislabeled specimens, sample contamination, and personnel competency (Table 5). This risk can be mitigated through adequate personnel training, the creation of appropriate procedure with emphasis on correct sample requirement and adherence to daily, weekly and monthly maintenance guidelines

External Quality control was also evaluated for the span of thirty-one (31) days for the determination of a customized plan to modify quality control frequency. It is used to assess instrument performance, the stability of cartridge kits and to save cartridge costs. Controls consist of normal, heterozygous and homozygous types. A total of 25 results were reviewed and

demonstrated no QC failure or within an acceptable performance, therefore the established customized external QC frequency will be set to perform every 31 days which is based on the College of American Pathologist (CAP) common checklist guideline, for every new lot of reagent kit, every new shipment of reagents and for any needed troubleshooting purposes. Furthermore, the implementation of a quality assessment program will review all activities mentioned in the IQCP and provide corrective actions, as needed, in order to measure its effectiveness and modification in the case of new errors that are detected or identified. (Table 7)

TABLE 5 RISK ASSESSMENT SUMMARY

Possible Sources of Error	Frequency of Occurrence	Phase	Severity	Risk Acceptable Y/N	Mitigations
1.0 Specimen					
1.1 Mislabeled sample	Probable	Pre-analytical	Serious	N	Delta check, history
1.2 Collection/Container/Volume	Occasional	Pre-analytical	Minor	Y	Adhere to guidelines detailed in the procedure
1.3 Improper Storage	Occasional	Pre-analytical	Minor	Y	Rejection criteria-refer to SOP/ Training
1.4 Transport	Occasional	Pre-analytical	Minor	Y	Redraw sample- Remedial actions for mishandling
1.4 Integrity	Occasional	Analytical	Minor	Y	Repeat/redraw sample
2.0 Reagents					
2.1 Receiving and Storage	Occasional	Pre-analytical	serious	Y	24hour temperature monitoring
2.2 Expiration Dates	Unlikely	Pre-analytical	Minor	Y	Replace kit
2.3 Quality Control	Unlikely	Analytical	Negligible	Y	the system will not run/ visual inspection

Possible Sources of Error	Frequency of Occurrence	Phase	Severity	Risk Acceptable Y/N	Mitigations
3.0 Testing Personnel					
3.1 Training & Competency Assessment	Probable	Analytical	Serious	N	Annual Competency Evaluation and monitoring
3.2 Labeling & Cartridge handling	Occasional	Pre-analytical	Minor	Y	Direct observations of handling, Competency Evaluation
3.2 Proficiency Testing- Failure due to Instrument Malfunction	Unlikely	Analytical	Unlikely	Y	All PT failures are addressed and investigated
3.2.1 CAP PT procedure not followed/PT not handled in the same manner as a patient sample	Occasional	Analytical	Negligible	Y	Provision of instruction to testing personnel/ signed an attestation form
3.3 Staffing- Inadequate to perform testing	Unlikely	Analytical	Negligible	Y	Sample can be stored for 15 days w/ temp. monitoring

Possible Sources of Error	Frequency of Occurrence	Phase	Severity	Risk Acceptable Y/N	Mitigations
4.0 Test system					
4.1 Contamination	Unlikely	Analytical	Negligible	Y	Remove and; used new cartridge if necessary
4.2 System errors- Pressure, air bubbles, temperature, optical signal, communication	Occasional	Analytical	Negligible	Y	System checks; Follow maintenance procedure
4.3 Defective Cartridge	Occasional	Analytical	Negligible	Y	Visual Check, Repeat with new cartridge kit
4.5 Defective modules	Occasional	Analytical	Negligible	Y	Disable the module; Call Technical support
5.0 Environment					
5.1 Temperature/airflow/ humidity/ventilation	Unlikely	Pre-analytical	Negligible	Y	Appropriate environmental conditions maintained
5.2Sample/Amplicon Contamination	Unlikely	Pre-analytical	Serious	N	Procedure for proper decontamination
5.3 Maintenance	Unlikely	Pre-analytical	Negligible	Y	Criteria defined in procedure
5.4 Electric	Unlikely	Pre-analytical	Negligible	Y	Connected to UPS power supply
5.5 Defective Cartridge	Occasional	Analytical	Negligible	Y	Visual check, Internal control failure “Invalid”
6.0 Test Results					
6.1 Review results	Unlikely	post-analytical	Minor-Critical	Y	Auto-transmission of results/delta check
6.2 Providers Complaints/ Inquiries	Unlikely	post-analytical	Minor	N	Investigate/Review IQCP/Modify
6.3 Release of results	Unlikely	Post-analytical	Negligible to serious	N	Criteria defined in procedure

TABLE 6 SUMMARY OF QCP

Type of Quality Control	Frequency	Criteria for Acceptability
Testing of appropriate external QC before or concurrently when in use	• Each new lot • Each new shipment • Monthly • PM/software upgrade	Results are within specified QC range Any QC failure is investigated immediately, addressed by the QC CLS to QC Manager and/or Supervisor
Instrument Preventive maintenance	• Daily (each day of use) • Weekly and as needed • Monthly and as needed	Meet acceptable criteria as defined in the Cepheid maintenance section
Room Temperature check Refrigerator Temp check	24/7 continuous monitoring	20-25 degrees C 2-8 degrees C
Inter-instrument correlation	Every 6 months	Correlation of test result and External QC testing on both GeneXpert instrument
User training/Competency	• Each new user and users prone to errors Initial, 6 months and annual	Training checklist completed Pass all 6 elements of assessment
PT assessment survey- TPM	Two- Three times/year	>80% score

TABLE 7 SUMMARY OF QA ACTIVITY

Frequency	QA Activity
Daily	Daily review of the patient's result for reporting errors and/physician complaints. Investigate and initiate occurrence management report (OMR)
	Confirmation of successful result transmission. Review BRL
	Monitor Review queue for aberrant/unusual results.
Weekly	Audit GeneXpert PM charts for compliance
	Review any new lot or shipment QC, as applicable
Monthly	Review monthly QC Data. Initiate corrective action(s) and revise QCP when unexpected QC failures indicate an adjustment to the QC plan defined.
	Review Isensix temperature logs and perform corrective actions when indicated, appropriately documented.
	Review of all equipment maintenance/ monitoring logs according to SOP
	Review PT Scores and evaluate shift trends. Initiate corrective action as needed
	Perform regular training and user competency assessment based on protocols, modify if necessary
	Monitor pre-analytic quality indicators that address specimen handling and erroneous specimen labeling. Take corrective action needed
Annually	Review manufacturer’s instruction for any changes or updates
	Confirm current procedure are available to users
	Confirm procedure accuracy and clarity
As needed	Update procedures, training checklist and/or competency form
	Follow up on complaints; Investigates the root cause(s) of error correction
	Examine reasons for QC failures, PT failures and patient isolate reporting errors and address, as needed, in a new/updated risk assessment: 1) Has a new risk factor been identified? 2) Does this change the frequency of risk? 3) Does the risk change the potential severity of harm of patient?

COSTS SAVING

Preventive Maintenance cost- The elimination of seven (7) instruments that includes two (2) EZ instruments for DNA isolation, three (3) thermal cyclers for amplification and two (2) Tecan readers for sample detection will save an estimated amount of $10,000.00 per year for preventive maintenance cost and other additional expense for other instrumentation problems.

Turnaround Time (TAT)- GeneXpert Sample processing time is about thirty-two (32) minutes for each test. Patients sample results are verified each day of testing and are available within 24 hours compared to the current method that has 4-5 hours of processing time and 5 -7 days TAT.

Personnel Utilization and Risk of Injury- With the GeneXpert technology system, it utilizes only one (1) CLS equivalent to a 0.5 FTE who is performing a two (2) step process involving sample pipetting, dispensing into the cartridge and then loading it directly into the instrument module. With the current PCR method, the processing time is 4-6 hours requiring a 1.0 FTE. The process involves multiple users to perform the tests with three (3) different instruments causing an increased risk of personal injury due to a repetitive range of motion and potential manual entry errors that may affect patient management. Summary of comparison between GeneXpert and current method are detailed in a table form. (Table 8)

TABLE 8 COMPARISON OF GENEXPERT VS CURRENT PCR METHOD

Comparison	GeneXpert Technology	Current PCR Method
Actual sample DNA Isolation/	Not performed, occurs inside the kit	30 minutes per sample
DNA Amplification/Detection	Performed within the cartridge kit	4.5 hours
Reporting	Manually entered, interpreted and verified	Result automatically transmit and batch verified
Pipetting	2 step pipetting	12 step pipetting
Instrument used	1	3
Personnel needed	0.5 FTE /shift	1.0 FTE/shift
Turnaround time (TAT)	24 hrs	5 – 7 days
Staff injury (Repetitive pipetting)	Decreased/ rare	Increased/ high probability
Preventive Maintenance (# of instruments)	1 instrument per year	7 instruments per year

DISCUSSION

Thrombophilia is defined as an increased risk or tendency to develop blood clots as a result of predisposing factors that may be inherited or acquired. It is usually related to the abnormality of the clotting system of some individuals causing DVT or pulmonary embolism. The determination of both Factor V Leiden and PT20210 mutation using GeneXpert technology gives molecular testing a different perspective from usual standard PCR testing. The test is moderately complex, but the assay is a simple 2 step process that it can be performed by personnel with less background in molecular testing. It is a fast, single test assay that eases workflow and delivers same day test results to the clinicians. The technology allows flexibility to perform other tests for the benefit of better patient management and treatment.

With the approval of the laboratory medical director for the IQCP implementation, the risk assessment analysis reinforces the accuracy of test results thru the detection of possible sources of error throughout the three (3) phases of testing. It is enhanced by a customized quality control plan and a comprehensive quality assessment that will be reviewed and monitored as indicated in the QC and QA summary. All necessary corrective action will be applied and implemented to mitigate prevent failures and probable sources of error.

CONCLUSION

Given its heterogeneity of clinical expressions and still lack of gold standard of testing, the Cepheid GeneXpert technology will improve the management of individuals who have the greatest probability of having venous thromboembolism caused by the genetic mutations of Factor V Leiden and PT20210 variants. Result turnaround time is reduced to 24hrs, prevents potential staff injury from repetitive motion and promote cost saving for labor and instrument maintenance.

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